Summary tables for spleen size and blood counts from Taylor et al (follows these pages) compared with studies from the Wistar group[1-3]

Notes:

(i) Detailed comparison of blood counts between sites is not possible since different acquisition methods were used, and in some cases, different populations of cells were profiled. For example, we looked at monocyte and lymphocyte subsets, while Kinder did not.

(ii) Since we do not have the primary data from the Kinder and Middleton papers, we estimated spleen size and blood counts from figures. Thus we are unable to include statistical information here.

(iii) Each site used different wild type mice for each experiment, and so data from *Alox15*$^{-/-}$ mice needs to be compared with the relevant controls from that site. Thus, comparison between mice at different locations cannot be undertaken. For full statistical analysis of each experiment done separately and shown as bar charts, see the following pages, and the relevant studies from the Wistar group[1-3].

(iv) Blood counts are given as $10^3/\mu l$ for all populations, except for platelets and erythrocytes which are $10^6/\mu l$.

Table 1. Spleen sizes from all sites.

	Mean spleen size (g)							
	Alox15$^{-/-}$				Wild Type			
Age	Cardiff N7/N11	Erlangen N11	Berlin N7	Wistar* N11	Cardiff N7/N11	Erlangen N11	Berlin N7	Wistar N11
2-3 mo	0.083/-	0.1106	0.0871	0.26	0.089/-	0.1175	0.08	0.1
7 mo	0.1153/0.1042	-	0.1252	-	0.0949/0.0806	-	0.09	-
13 mo	-	-	0.1388	-	-	-	0.0839	-
17 mo	-	-	0.145	-	-	-	0.081	-
19 mo	-	-	0.16	-	-	-	0.113	-

*15% of spleens at Wistar at 2-3 mo had spleen sizes approx. 0.6 gm.

Table 2. Blood counts from all sites.

	Blood Counts	Age	Leukocytes/WBC	Erythrocytes	Neutrophils	Total lymphocytes	Total monocytes	Basophils
***Alox15*$^{-/-}$**	Cardiff N7	7 mo	9.155	10.4	0.955			
	Cardiff N11	7 mo	10.68	8.75	0.777			
	Wistar (estimated)	3-4 mo	2	8.8	0.3	2	0.05	0.09
	Wistar (estimated)	7 mo	2.5	8.8	0.4	1.8	0.05	0.06
Wild Type	Cardiff N7 controls	7 mo	11.4	10.05	1.805			
	Cardiff N11 controls	7 mo	12.9	9.4	1.28			
	Wistar (estimated)	3-4 mo	9	11	0.3	8	0.24	0
	Wistar (estimated)	7 mo	9.8	11	1.1	9	0.3	0

	Blood Counts	Age	Reticulocytes	Eosinophils	Ly-6B+ monocytes	Ly-6B- monocytes	CD4+ T-cells	CD8+ T-cells	CD19+ B-cells	Platelets
***Alox15*$^{-/-}$**	Cardiff N7	7 mo		0.212	0.465	0.856	0.552	0.735	4.433	1.327
	Cardiff N11	7 mo		0.209	0.284	0.479	1.1014	1.037	6.101	1.115
	Wistar (estimated)	3-4 mo	450							
	Wistar (estimated)	7 mo	350							
Wild Type	Cardiff N7 controls	7 mo		0.291	0.622	0.85	0.692	0.855	5.126	1.19
	Cardiff N11 controls	7 mo		0.203	0.338	0.432	1.107	1.183	7.519	1.229
	Wistar (estimated)	3-4 mo	350							
	Wistar (estimated)	7 mo	350							

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Supplementary Data (Taylor et al)
Development of myeloproliferative disease in 12/15-lipoxygenase deficiency.

Summary

Recent studies in Philadelphia (Wistar Institute) indicate that 12/15-lipoxygenase (LOX)-deficiency in (C57BL/6, N11) mice leads to substantial splenomegaly (from 2 months), altered splenic architecture, severe myeloproliferative disease, significant peripheral leukopenia and elevated mortality. We compared three independent colonies (Cardiff, Berlin, Erlangen) of the same knockout mice (N7/N11) with the Wistar mice and found the following differences: i) Young mice (Cardiff, Berlin, Erlangen) showed no splenomegaly, although a small progressive increase in spleen size was evident between 7 (Cardiff, Berlin) and 19 (Berlin) months; ii) there was no increase in 1 yr mortality (Cardiff, Berlin); iii) there was only a minor loss of peripheral leukocytes by 7 months (Cardiff); iv) splenic architecture was preserved at 7 and 19 months (Cardiff, Berlin). Additionally, the Jackson Laboratories report no obvious health problems by 7 months. This mild phenotype indicates that 12/15-LOX-deficiency is not sufficient for severe myeloproliferative disease.

Background

12/15-lipoxygenase (LOX) is a lipid metabolizing enzyme predominantly expressed by cells of the monocyte/macrophage lineage. Its detailed biological function is unclear, but it was reported to act as a tumour suppressor, through regulating interferon consensus sequence binding protein (ICSBP)/interferon regulatory factor-8 (Irf8) [1-3]. In 12/15-LOX-deficient (*Alox15*$^{-/-}$) mice, moderate splenomegaly (doubling of spleen size) with 100 % penetrance was observed at 10 - 12 weeks, along with profound blood leukocytosis and basophilia [3]. Subsequently, the defect was characterized as primarily due to loss of haematopoeitic stem cell function, with profound reduction in white cells accounted for by a decrease in lymphocytes, monocytes and eosinophils in non-moribund mice [2]. A small decrease in erythrocytes was noted, but there was an increase in basophils and no change in neutrophils [2]. Furthermore, the mice exhibited an enhanced mortality, with approximately 25 % mortality by 12 months [3]. Strikingly, up to 15 % of these animals also became moribund after this young age, and were considered to be in blast crisis. These had grossly enlarged spleens at up to 6-fold normal masses [3]. Since approximately 1:6 mice become moribund after 10 - 12 weeks of age, the defect is significant and would be expected to be noted in colonies of these mice housed at different centres.

12/15-LOX-deficient mice were generated around 1993 on a 129S2 background by Funk, in Nashville. They were backcrossed against C57BL/6 mice for 7 generations (N7), then moved to UPenn (by Funk) in 1996, where they were re-derived. From 1997-2003 they were used for atherosclerosis studies at UPenn, as well as being freely provided to the research community, including Berlin (Kühn) in 1998. An initial characterization of blood cells showed no significant changes to blood cell populations including white cells, red cells, neutrophils and monocytes [4]. The colony bred in Berlin was infected by mouse hepatitis virus, so aseptic embryo transfer was carried out at Charles River (Germany) in 1999. The new colony was maintained at Charles River under specific pathogen free (SPF) conditions until June 2001, when it was transferred back to the Berlin laboratory. From the SPF colony, 3 breeding pairs were sent to Cardiff (O'Donnell) in 2004 where they have been continuously bred since.

In 1994 - 1998 the UPenn colony was further backcrossed to N11 by Funk and then provided to Jackson Laboratories through which they are commercially available. After moving to Kingston, Ontario in 2004, a new colony was established from N11 founders from JAX (Funk). These were mated with *ApoE*$^{-/-}$ to generate double knockout mice, which were are reported to show only a very small and non-significant increase in spleen size by 20 - 24 weeks of age [5]. Studies at the Wistar Institute used N11 mice obtained from

Jackson Laboratories initially (housed in the facility from 8 weeks of age when they were purchased) and thereafter, interchangeably with a colony established from those mice onsite [3]. The data in this paper is from the Cardiff and Berlin colonies, as well as one from Erlangen (Krönke) generated from N11 founders purchased from Jackson Laboratories in 2007.

When the myeloproliferative disorder (MPD) was reported, it was suggested that other laboratories may not have noted this because backcrossing to N11 may make the disorder more pronounced on the C57BL/6 background [3]. Seven generations of backcross would theoretically make the *Alox15*$^{-/-}$ mice 99.21 % genetically identical to wild type C57BL/6, with 4 further rounds of backcrossing increasing this to 99.95 %. Thus, the mice are already substantially genetically related to C57BL/6. To address whether this was responsible, we continued the backcross of our N7 colony to N11 (Cardiff), and compared blood counts and spleen size in both strains, in both young and aged mice. Comparison of these mice with colonies at Erlangen, Berlin, Kingston, and Jackson Laboratories indicate that deficiency of 12/15-LOX alone is not sufficient for development of the profound hematological phenotype, observed in the Wistar Institute colony. Although some features of very mild disease are indicated (only a minor elevation in spleen size), alterations in blood counts, mortality rates and spleen size are not consistent with those reported at Wistar.

Experimental Procedures

Housing and heath status information.

Health status reports are at the end of this supplement.

1. Cardiff. All animal experiments were performed in accordance with the United Kingdom Home Office Animals (Scientific Procedures) Act of 1986. *Alox15*$^{-/-}$ mice and wild-type male C57BL/6 mice (25–30 g) from Charles River, UK, were kept in constant temperature cages (19 - 23 °C) and given free access to water and standard chow. *Alox15*$^{-/-}$ mice were bred in an isolator before being transferred after weaning to conventional cages. Health status information is given below. Mice were sacrificed by inhalation of 70 % CO_2. Blood was harvested using cardiac puncture into EDTA then mice were dissected to recover liver, spleen and heart. In summary, throughout 2010-2011, the colony was negative for 19 viruses, 3 parasites and 15 bacterial strains. Positive results were obtained for mouse norovirus (MNV), intestinal protozoa and helicobacter. Treatments were initiated for protozoa (2 weeks antibiotics), and negative results obtained thereafter.

2. Berlin*: Alox15*$^{-/-}$ mice (N7) were bred in the local animal house of Charité. Wild-type C57BL/6 controls were purchased from Charles River, Germany, at the age of 8 weeks and maintained in our local animal house until they reached the desired age. The animal experiments at Charite were approved by the corresponding commission of the local government (Landesamt für Gesundheit und Soziales Berlin), approval No. T0437/08. Mice were given free access to water and standard chow diet and were kept at an artificial night and day rhythm. Mice were sacrificed by cervical distortion. Health status information is given below. In summary, during the past 18 months, the colony was negative for the 12 viruses tested, for most of the tested bacteria, mycoplama and fungi. Positive results were obtained in March 2011 for helicobacter and in 2010 for Pasteurellaceae. In addition, animals were tested for 8 parasite infections. Mice were dissected to recover liver, spleen and heart.

3. Erlangen*: Alox15*$^{-/-}$ mice were purchased from Jackson Laboratories (ME, USA), housed in conventional cages, and sacrificed by cervical dislocation. Animal experiments were approved by the government of Mittelfranken. Mice were housed in the animal facility of the University of Erlangen-Nuremberg. Health screens were performed on a regular basis.

The health history af the last 4 years shows positive results for mouse norovirus (MNV), helicobacter, Staphylococcus, Pseudomonas, Entamoebia, Chilomastix and Trichomonas.

Genotyping. All *Alox15*$^{-/-}$ mice included in this study were genotyped by genomic PCR. For this purpose genomic DNA was prepared from the ear or tail snips of the mice using the INVITEK Invisorb Spin Tissue Kit and genotypes were verified using the following primer combination. Neo primer-1: 5' CTT GGG TGG AGA GGC TAT TC 3', neo primer-2: 5' AGG TGA GAT GAC AGG AGA TC 3'; exon3 primer: 5' CTG GGT TGA AGA CTC TCA AGG 3'; exon4 primer: 5' CGA AAT CGC TGG TCT ACA GG 3'. Two primers anneal in the neomycin cassette that was introduced into the gene during the knockout strategy. One primer anneals in exon 3 the other one in exon 4. For wild-types, a PCR product of 392 bp was expected whereas homozygous knockout individuals were characterized by a PCR product of 715 bp. For heterozygous knockout mice, both bands were observed with similar intensity. For amplification, 50 ng genomic DNA dissolved in 17 µL sterile water was employed. This solution was heated for 10 min at 99° C and then cooled on ice for 5 min. Then 33 µL of the PCR master mix was added and genomic PCR was performed. The master mix consisted of 2 µL of the four primer dilutions and 25 µL of the Biomix-red 2-fold PCR buffer (BIOLINE). The complete PCR sample (50 µL) was heated for 8 min at 94° C and then a two phase amplification period was started. The 1st phase involved 12 amplification cycles each consisting of a denaturation phase (20 s at 94 °C), an annealing phase (30 s at 64 °C) and a synthesis phase (35 s at 72 °C). The 2nd phase involved 25 amplification cycles each consisting of a denaturation phase (20 s at 94 °C), an annealing phase (30 s at 58 °C) and a synthesis phase (35 s at 72 °C). Finally, an isothermic post-run (10 min at 72 °C) was carried out and the samples were stored at 10 °C. The PCR products were analyzed on a 2 % agarose gel. The genotypes of the mice used in these studies were verified *postmortem* using this protocol.

Backcrossing of the Cardiff N7 colony to N11. Female N7 *Alox15*$^{-/-}$ mice were mated with wild-type C57BL/6 mice to generate heterozygous deficient animals that were selected after genotyping (see above) for further rounds of backcrossing. N11 heterozygous-deficient mice were bred together to generate homozygous *Alox15*$^{-/-}$ mice that were identified using PCR and used as the founders of the new N11 colony.

Measurement of blood cells using flow cytometry in Cardiff mice. Cardiac blood (<1 ml) was harvested directly into 100 µl of 0.5 M EDTA and then kept on ice until analysis. For total leukocyte, platelet and erythrocyte counts, 10 µl aliquots of whole blood were transferred to separate tubes and incubated at 4 ^{o}C for 20 min with the cell permeant DNA dye Draq5 (5 µM; Biostatus) in 100 µl final volume. A 10 µl of aliquot of this Draq5 stained whole blood (equivalent to 1 µl of blood) was diluted > 30-fold in 1% formaldehyde in PBS and fluorescent flow-cytometric counting beads (Bangs Labs) were added before mixing and acquisition on a CyAn ADP analyser (3 laser; Beckman-Coulter). Platelets and nucleated leukocytes were discriminated from erythrocytes by their characteristic FSC/SSC profile and uptake of Draq5 respectively (see below). For leukocyte subset identification, 10 µl of whole blood was added to 40 µl of FACS block (4 µg/ml 2.4G2 anti-FcγRII/III, 5 % heat-treated rabbit serum, 5 mM EDTA, 2 mM NaN_3 in PBS) and incubated for 20 minutes at 4 ^{o}C. After blocking, the required directly conjugated antibodies were added in an additional 50 µl of FACS block and incubated for 1 hour at 4^{o}C. The cells were then pellet at 350 x *g* for 4 minutes and resuspended in ACK lysis buffer (0.15M NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA) for 3 minutes at room temperature. Three such lysis steps were completed before resuspension of the erythrocyte-cleared leukocytes in PBS, fixation in 1% formaldehyde in PBS and acquisition on the CyAn ADP analyser. All flow-cytometric data was analysed with Summit software (Beckman-Coulter). The

antibodies used were: CD11b-FITC (homemade); Ly-6B.2-phycoerythrin, F4/80-allophycocyanin and CD19-allophycocyanin (AbD Serotec); CD8a-FITC (Caltag); CD4-PE (Sigma). Leukocytes were identified as follows: Monocytes were identified as $CD11b^{+}$, SSC^{low}, $F4/80^{+}$ cells, which differentially expressed Ly-6B.2 [6]; Neutrophils were identified as $CD11b^{+}$, SSC^{high}, $Ly\text{-}6B.2^{+}$, $F4/80^{-}$ cells[7]; eosinophils were identified as $CD11b^{+}$, $SSC^{very\ high}$, $F4/80^{+}$, $Ly\text{-}6B.2^{-}$[7]; lymophocytes were identified by the expression of CD4 and CD8 (T cells) and CD19 (B cells).

Tissue processing for spleen morphology. Spleens were fixed in 10 % neutral buffered formal saline before embedding in paraffin. Sections of 5 μm thickness were cut using a microtome (Surgipath). Before staining, sections were de-waxed and rehydrated through xylene/ethanol. Harris haematoxylin (BDH) and eosin (Thermo Scientific) (H&E) staining was followed by ethanol/xylene dehydration and mounting in DPX (Sigma Aldrich). Cardiff spleens were stained for haematoxylin only. White-pulp area and 'roundness' (roundness = $perimeter^2/4\pi$ x Area) were determined on H&E stained sections of spleen by manual delineation of the areas of white-pulp and analysis of area and perimeter by QWin Software (Leica). Data were determined from between 27 and 39 individual white-pulp regions in a section of whole spleen (Cardiff, N7) or from between 14 and 49 white-pulp regions from sections of spleen segments (Berlin).

Results.

Young (2 - 3 months) N7 Cardiff and N11 Erlangen mice show normal spleen size, but aged (7 months) N7 or N11 Cardiff mice show small differences. Mixed gender groups of young wild-type or N7 *Alox15*$^{-/-}$ mice (2 - 3 months) from Cardiff showed no significant differences in spleen or liver weight (Fig 1 A, B). Similarly, mixed gender young (2 – 3 months old) wild-type or N11 *Alox15*$^{-/-}$ mice from Erlangen showed no significant differences in spleen weight (Fig 1 C). Mixed gender groups of aged (7 months old) wild type or Cardiff N7 *Alox15*$^{-/-}$ mice showed very small but significant differences in spleen and liver mass (Fig 1 D,E). Also, there was a small but significant decrease in total white cells, but no change in red cells or platelets (Fig 1 F-I). The decrease in white cells was reflected by small, but not significant, decreases in most populations, including neutrophils, monocytes ($Ly\text{-}6B^{+}$ subset), T-cells and B-cells (Fig 1 J,K,M-R). The only significant difference was for eosinophil counts (Fig 1 L). No signs of illness were apparent throughout the maintenance of these mice at Cardiff. After backcrossing a further 4 generations in Cardiff, mixed gender groups of wild type or N11 *Alox15*$^{-/-}$ mice were sacrificed at 7 months, and spleens, liver and heart weights and blood counts recorded. Results were very similar to 7 month old N7 mice (Fig 2 A-M). Spleen weight showed a small but significant increase with 12/15-LOX deficiency, but liver and heart was unchanged (Fig 2 A-C). Also, there was a small decrease in total white cells, but this was not significant, and no change in red cells or platelets (Fig 2 D-F). Again, the trend for a decrease white cell count was reflected by minor but non-significant decreases in specific cell types (Figure 2 H, J-M). In this study, only the neutrophil count was significantly different (Figure 2 G). As for N7 mice, no signs of illness or elevated death rates were noted in this colony.

Aged N7 Berlin mice (from 2 to 19 months) showed a similar modestly increased spleen size, but no sign of elevated death rate or illness. Spleens from mixed gender older *Alox15*$^{-/-}$ mice (N7 Berlin) began to show significant changes, at 13 months, although similar to Cardiff mice, small changes were evident at 7 months (Fig 2 N). However, no spleens approached previously published mean weights (approximately 0.6 g for 'moribund' *Alox15*$^{-/-}$ mice, ref paper), with the largest 19 month old *Alox15*$^{-/-}$ spleen

recorded at 0.285 g (compared to the largest 19 month old wild type spleen of 0.227 g). Furthermore, no mice appeared moribund or showed any overt signs of ill health. Examination of historical data showed that out of 75 mixed gender *Alox15*$^{-/-}$ Berlin mice, 5 died by 5 months and a further 2 by 17 months. The reason for death was not determined at the time, however this gives a death rate of 6.6 % and 9.3 % at 12 and 17 months, respectively.

Berlin and Cardiff mice show no alterations in splenic architecture. Spleens from aged (7 month) wild type and *Alox15*$^{-/-}$ Cardiff mice showed similar pattern and distribution of white pulp regions to the wild type controls (Figure 2 O,P). Similarly, spleens from 19 month old male animals in Berlin exhibited no difference in white pulp area and roundness between the two genotypes (Figure 2 Q,R).

Discussion

A myeloproliferative disorder (MPD) was recently described in colonies of *Alox15*$^{-/-}$ mice housed in Philadelphia (Wistar Institute) [1-3]. The phenotype reportedly shows 100 % penetrance and 15 % are reported as moribund after 2 - 3 months weeks of age [3]. It was suggested that other groups may not have noticed the phenotype since some colonies had only been backcrossed from the 129S2 substrain onto C57BL/6 for 7 generations (N7). As the mice have been in existence for approximately 18 years with no apparent signs of ill health, we were prompted to examine for this phenotype both pro- and retro-spectively in as many other laboratories as possible. Overall, our data indicates that other colonies of these mice show a mild phenotype that is inconsistent with that reported for the Wistar Institute mice. Specifically, apparently healthy 10 - 12 week old mice do not show a doubling in spleen size, or profound alterations in blood cells. Nor do approximately 15 % of them progress to what appears to be accelerated CML and blast crisis. Consistent with our data, a previous study from Kingston (Funk) found that 20 - 24 week old *Alox15*$^{-/-}$ mice backcrossed with ApoE$^{-/-}$ mice displayed only small increases in spleen size (similar to ours), when compared with ApoE$^{-/-}$ controls, and only at older ages [5]. It is noteworthy that even up to 19 months of age, no mice in our colonies appeared outwardly ill, thus none could be classified as moribund at any age.

Young Cardiff N7 *Alox15*$^{-/-}$ mice (2-3 months) showed no change in spleen size with a mean mass of 0.083 g and the largest 0.1 g [8]. In contrast, the Wistar Institute mice at this age already showed significant increases, with 85 % having a mean spleen mass approaching 0.2 g, approximately double the normal level [3]. In that study, the remaining 15 %, described as moribund, displayed a mean spleen size of approx 0.6 g, with approximately 15 % of these spleens being >0.75 g. No spleens of this size were ever observed in mice of any age in Berlin or Cardiff, Erlangen, or in older mice in Kingston (Figs 1,2 and data not shown). Older Cardiff mice (7 months) (N7 or N11) showed small but significant increases in spleen size (mean 0.115 g and 0.104 g for N7 and N11 mice, respectively) (Fig 1C, 2A). Similarly, Berlin N7 mice also showed mild splenomegaly, that was not apparent until at least 7 months of age or older (Fig 2 N). Beyond this, splenomegaly increased further, but never achieved individual masses greater than 0.285 g by 19 months of age. Notably, no ill or moribund mice were ever noted in Cardiff, Berlin, Erlangen or Kingston. Overall the data indicates that these colonies are considerably healthier, and that backcrossing to N11 *per se* does not explain the profound splenomegaly observed in the Wistar colony from 10 - 12 weeks. Furthermore, mortality rates in Berlin (6.6 % and 9.3 % at 12 and 17 months, respectively) are similar to previously published values of approx 6 % and 15 % for C57BL/6 mice, in contrast to the 25 % at 12 months mortality reported for the N11 *Alox15*$^{-/-}$ mice at Wistar [3 9]. Lastly, we contacted Jackson Laboratories, who report that they have not observed sick/moribund

mice through to their breeding retirement age of 6-7 months (Peter Kelmenson, Personal Communication).

Blood counts from N7 and N11 Cardiff mice were very different to blood counts of equivalent age N11 Wistar mice (7 months), previously published in [2] as Supplementary Figure 1. Specifically, the Wistar Institute colony (non-moribund mice) showed considerably larger decreases, including a 75 % drop in total white cells and losses of red cells, lymphocytes and monocytes of 24 %, 82 %, 83 % respectively. Unexpectedly, we noted a significant fall in neutrophil numbers in the Cardiff N11 colony with a similar trend in the N7 colony, while the Wistar Institute colony appears to maintain these cells at normal or elevated levels (Figs 1K, 2G [2]). These data indicate that the Cardiff N7 and N11 mice do not show the profound changes in blood cells observed at Wistar, nor do the subtle changes observed in Cardiff follow the trends of those observed at Wistar. As before, the effect of backcrossing to N11 fails to explain this discrepancy. Of note, in 1996, it was reported that young (8 week) old *Alox15*$^{-/-}$ mice (UPenn, Funk) showed normal blood counts for white cells, neutrophils, monocytes, erythrocytes, reticulocytes and platelets [4].

The phenotype reported in the Wistar mice is consistent with MPD, with moribund mice showing features in-keeping with myeloid blast transformation of that condition to acute myeloid leukemia (AML). Splenomegaly is a recognised clincal feature of all MPDs. Basophilia is more particularly associated with CML, although in the absence of the pathognomonic *Bcr-abl* gene fusion, the *Alox15*$^{-/-}$ mice would not be considered to have true CML. To provide a tractable model for the future study of mechanisms of transformation of MPDs and CML, development of a full understanding of why the severe Wistar phenotype is not common to all colonies is important. In particular, eludication of why only mice from that colony show transformation to acute leukaemia (accelerated phase and blast crisis) from what appears to be a mild disorder elsewhere may lead to the identification of molecular therapeutic targets for future clinical intervention.

Our data suggests that an additional factor may be required to initiate the profound defect seen in the Wistar mice. Since infection is one potential difference between the colonies, we compared health status results for all four. Both the Wistar Institute and the Berlin colonies were negative for all tested viruses, whereas the Cardiff and Erlangen mice were positive for norovirus. This is the most common viral pathogen in laboratory mice, but is not known to cause any associated tissue pathology in immunocompetent strains following initial infection [10 11]. Berlin, Erlangen and Cardiff mice had helicobacter, and while some strains can cause hepatitis, gastritis and liver tumours, these were never noted in our mice. The Wistar Institute mice were not tested for microbial infection (only tested for 15 viral agents compared to 40, 33 and 35 viral, parasite and microbial tests in Cardiff, Erlangen and Berlin, respectively). The Jackson Laboratories colony tested consistently negative for all 43 infectious agents studied. Full information on health status is given at the end of this supplement. We cannot exclude the possible presence of other unidentified pathogens in the Wistar Institute colony that may influence the health status of the animals, but there are no obvious consistent differences when considering the infectious agents screened.

A second potential explanation for these discrepancies could be an isolated genetic alteration. Although all mice were ultimately derived from the same original knockout strain, the colonies have been through distinct breeding programs that could have resulted in the acquisition in some mice of a specific alteration that modifies or causes the phenotypic differences.

In summary, MPD, elevated death rates and splenomegaly observed in the Wistar colony from 2 months of age were not consistent throughout the colonies we tested. Instead, only minor increases in splenic dimensions and equivocal reductions in white cell counts were found in more aged mice, explaining why this phenomenon has not been

observed at other sites. Hence the underlying mechanism leading to development of an accelerated 'CML-like disease' and blast crisis in Wistar Institute mice warrants further study.

Acknowledgements.
We gratefully acknowledge detailed discussions and input from Ellen Puré and colleagues Wistar Institute, including health status information for the colony.

Funding Sources.
Funding is gratefully acknowledged from Wellcome Trust (VBO). CDF is funded by the Canadian Institutes of Health Research and is holder of a Canada Research Chair in Molecular, Cellular and Physiological Medicine and Career Investigator of the Heart and Stroke Foundation of Ontario. P.R.T. is the recipient of a Medical Research Council (UK) Senior Research Fellowship (G0601617).

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Figure Legends

Figure 1. Organ masses and blood counts for young (2-3 month) and aged (7 month) N7 Cardiff *Alox15*$^{-/-}$ mice show only small changes versus wild type C56Bl/6. Wild type and *Alox15*$^{-/-}$ mice were sacrificed using CO_2, and blood obtained using cardiac puncture. Organs were harvested and weighed. *Panels A,B. Spleen and liver weights show no change from wild type mice at 10-12 weeks* (n = 8 per strain, mean ± SEM). This data was previously included as part of a figure in [8]. *Panel C. No change in spleen weights in young (2-3 month Erlangen mice*. Organs were harvested and weighed (n = 9 wild type and 11 *Alox15*$^{-/-}$, mean ± SEM) *Panels D,E. Organ weights for 7 month old Cardiff N7 mice show small changes in spleen weight*. Organs were harvested and weighed (n = 24 wild type and 24 *Alox15*$^{-/-}$, mean ± SEM). *Panels F-R. Blood cell flow cytometry data for Cardiff F7 aged mice.* Blood cells were determined using flow cytometry as described in Materials and Methods (n = 24 per strain, mean ± SEM). Panels F-I show representative flow-cytometric plots (F) and quantification of leukocytes (G), erythrocytes (H) and platelets (I). Panels J-N show representative flow-cytometric plots for the identification of myeloid cells (J), and quantification of neutrophils (K), eosinophils (L), and Ly-6B^{+} (M) and Ly6B^{-} monocytes (N). Panels O-R show representative flow-cytometric plots for the identification of lymphocytes (O) and quantification of CD4^{+} (P) and CD8^{+} (Q) T cells and CD19+ B cells (R).

Figure 2. Organ masses and blood counts for aged (7 month) N11 Cardiff *Alox15*$^{-/-}$ mice show only small changes versus wild type C56BL/6, but splenomegaly progresses with age (Berlin N7). Wild type and *Alox15*$^{-/-}$ mice were sacrificed using CO_2, and blood obtained using cardiac puncture. Organs were harvested and weighed. *Panels A-M. Organ weights and blood counts for 7 month old mice show small changes in spleen weight and, apart from neutrophils, only non-significant trends towards reduced white cell populations*. Blood cells were determined using flow cytometry as described in Materials and Methods (n = 4 wild type and 19 *Alox15*$^{-/-}$, mean ± SEM). *Panel N. Progressive splenomegaly occurs with age in the Berlin N7 colony*. Mice were sacrificed at the indicated ages and spleens weighed. Number of mice in each group is shown on the bar (mean ± SEM). Panel O shows representative spleen H&E staining of 7 month old wild type and *Alox15*$^{-/-}$ N11 mice (n = 4 per group). The areas of white pulp were marked on photomicrographs and the area (Panel P, left) of the regions was determined using QWin Software. Panel P shows a numerical representation of roundness (Panel P, right) of the white pulp region determined from the Area and perimeter (both determined in QWin) as described in the materials and methods. Panels Q and R show similar representative H&E staining (Q) and Area (R, left) and roundness (R, right) determinations for 19 month old wild type and *Alox15*$^{-/-}$ N7 mice (n = 3 per group) from the Berlin colony.

Figure 1

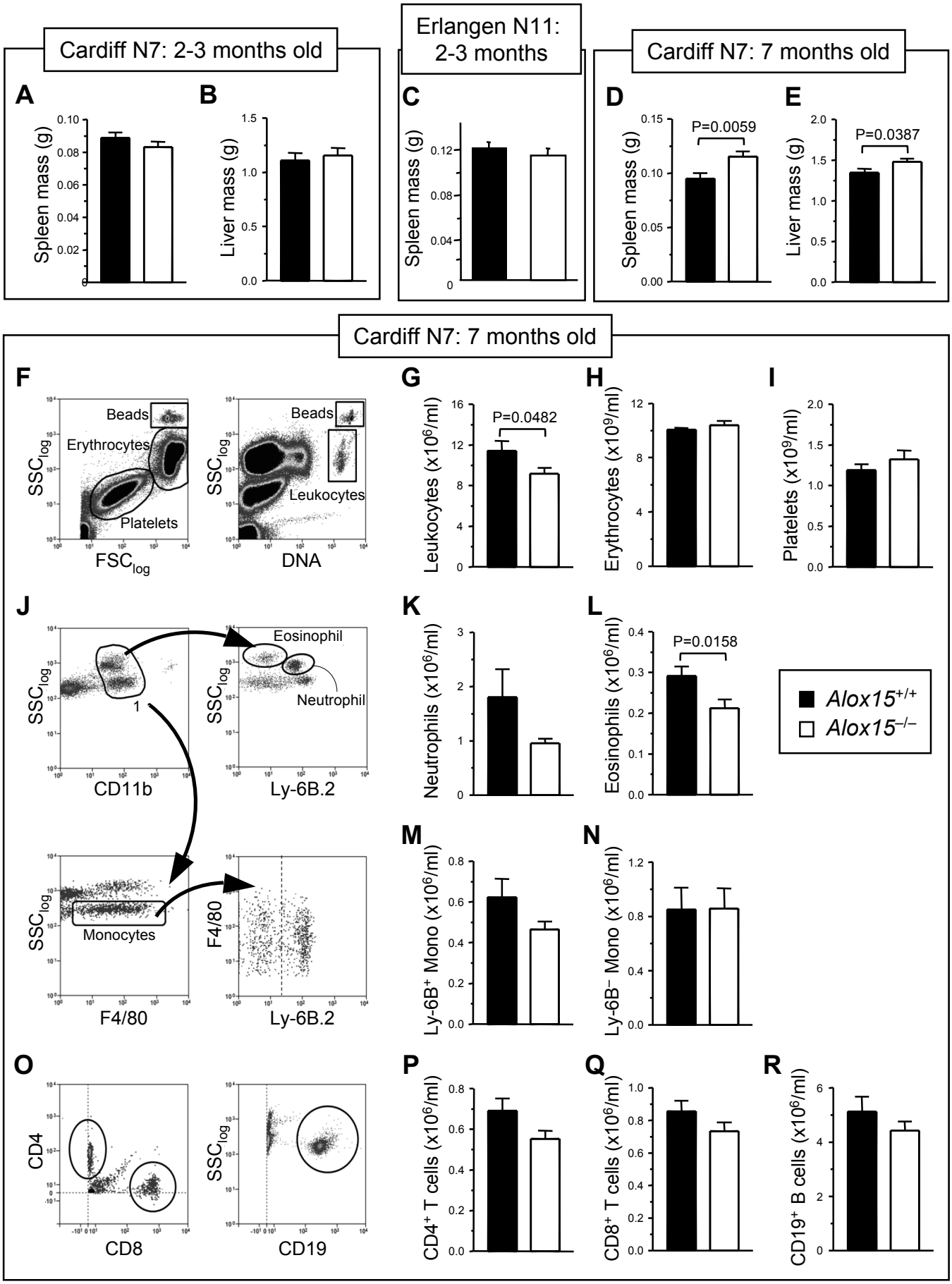
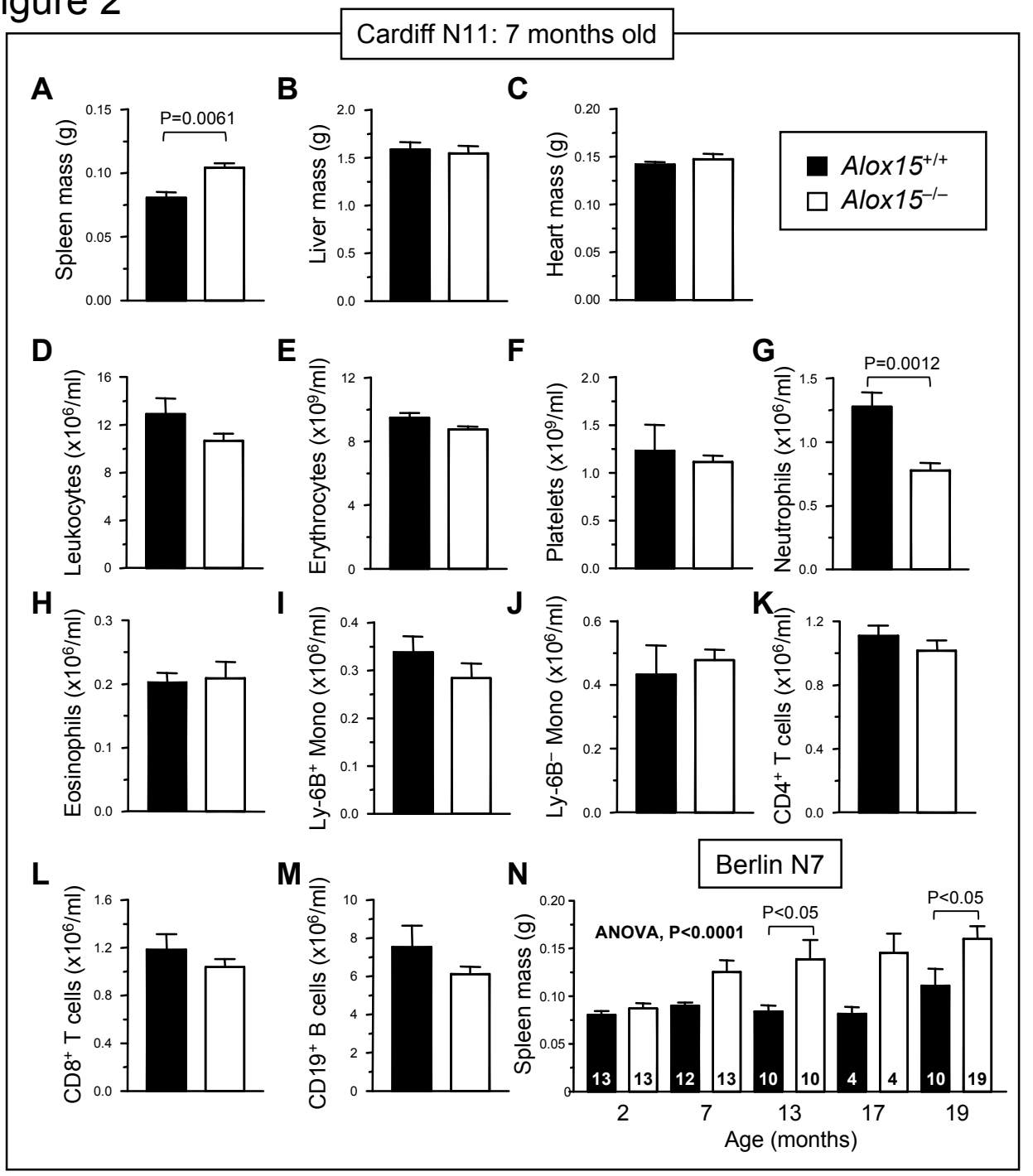
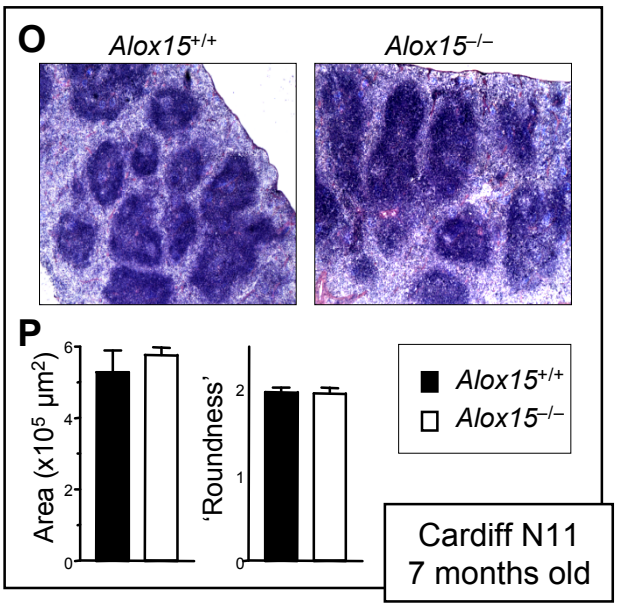
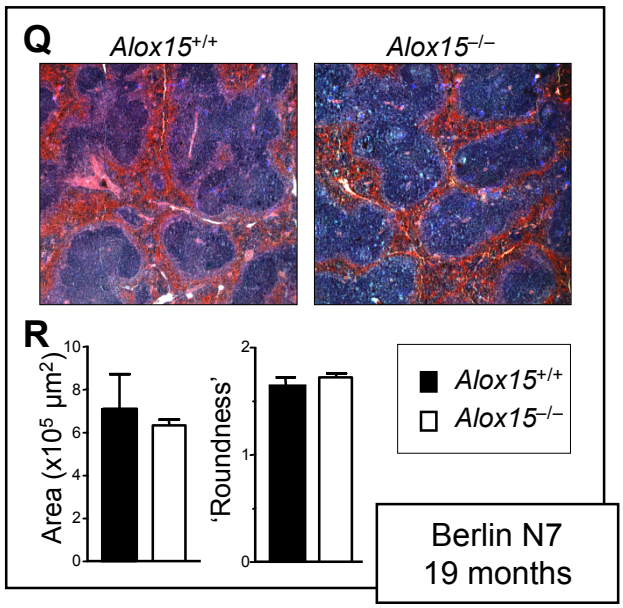

Cardiff N7: 2-3 months old
Erlangen N11: 2-3 months
Cardiff N7: 7 months old
A
Spleen mass (g)
B
Liver mass (g)
C
Spleen mass (g)
D
Spleen mass (g)
P=0.0059
E
Liver mass (g)
P=0.0387
Cardiff N7: 7 months old
F
Beads
Erythrocytes
Platelets
SSC log
FSC log
Beads
Leukocytes
DNA
G
Leukocytes (x10⁶/ml)
P=0.0482
H
Erythrocytes (x10⁹/ml)
I
Platelets (x10⁹/ml)
J
1
CD11b
Eosinophil
Neutrophil
Ly-6B.2
Monocytes
F4/80
F4/80
Ly-6B.2
K
Neutrophils (x10⁶/ml)
L
Eosinophils (x10⁶/ml)
P=0.0158
Alox15+/+
Alox15−/−
M
Ly-6B⁺ Mono (x10⁶/ml)
N
Ly-6B⁻ Mono (x10⁶/ml)
O
CD4
CD8
SSC log
CD19
P
CD4⁺ T cells (x10⁶/ml)
Q
CD8⁺ T cells (x10⁶/ml)
R
CD19⁺ B cells (x10⁶/ml)

Figure 2


Cardiff N11: 7 months old
A
Spleen mass (g)
P=0.0061
B
Liver mass (g)
C
Heart mass (g)
Alox15+/+
Alox15–/–
D
Leukocytes (x10⁶/ml)
E
Erythrocytes (x10⁹/ml)
F
Platelets (x10⁹/ml)
G
Neutrophils (x10⁶/ml)
P=0.0012
H
Eosinophils (x10⁶/ml)
I
Ly-6B⁺ Mono (x10⁶/ml)
J
Ly-6B⁻ Mono (x10⁶/ml)
K
CD4⁺ T cells (x10⁶/ml)
L
CD8⁺ T cells (x10⁶/ml)
M
CD19⁺ B cells (x10⁶/ml)
Berlin N7
N
Spleen mass (g)
ANOVA, P<0.0001
P<0.05
P<0.05
13 13 12 13 10 10 4 4 10 19
2 7 13 17 19
Age (months)


O
Alox15+/+
Alox15–/–
P
Area (x10⁵ µm²)
'Roundness'
Alox15+/+
Alox15–/–
Cardiff N11
7 months old


Q
Alox15+/+
Alox15–/–
R
Area (x10⁵ µm²)
'Roundness'
Alox15+/+
Alox15–/–
Berlin N7
19 months

Health status information for *Alox15*$^{-/-}$ at Jackson Laboratories.

The Jackson Laboratory
Leading the search for tomorrow's cures

Repository
Animal Health Report

AREA: AX-12 **Report # 1011AX12**

AX-12 IS OPERATED AS A:

◉ **Maximum Barrier** ○ **High Barrier** ○ **Standard Barrier**

Please consult our website for descriptions of our Barrier Levels.

ORGANISMS EXCLUDED FROM ALL BARRIERS (SHIPPING STOPPED) -
If any of these organisms are found in any Repository area, all shipments from the area are suspended and customers are notified.

Organism	Sample Tested	Test Method	Oct 17 '11	Sep 5 '11	Jul 25 '11	Jun 13 '11	Previous 12 months
VIRUSES							
Ectromelia virus	Serum	MFI	0/16	0/15	0/16	0/14	0/112
GDVII (Theiler's) virus	Serum	MFI	0/16	0/15	0/16	0/14	0/112
Hantaan virus	Serum	ELISA	0/16	0/15	0/18	0/14	0/124
K virus	Serum	ELISA	-	0/15	-	-	0/14
Lactic dehydrogenase elevating virus	Serum	Enzyme	-	-	-	-	0/10
Lymphocytic choriomeningitis (LCMV)	Serum	ELISA	0/16	-	-	0/14	0/56
Mouse adenovirus (MAV)	Serum	MFI	-	0/15	0/16	0/14	0/70
Mouse cytomegalovirus (MCMV)	Serum	MFI	0/16	0/15	0/16	0/14	0/112
Mouse hepatitis virus (MHV)	Serum	MFI	0/16	0/15	0/16	0/14	0/112
Mouse minute virus (MMV)	Serum	MFI	0/16	0/15	0/15	0/14	0/112
Mouse norovirus (MNV)	Serum	ELISA	0/16	0/15	0/16	0/14	0/112
Mouse parvovirus (MPV)	Serum	ELISA	0/16	0/15	0/16	0/14	0/112
Mouse thymic virus (MTV)	Serum	IFA	-	0/15	0/16	-	0/52
Pneumonia virus of mice (PVM)	Serum	MFI	0/16	0/15	0/16	0/14	0/112
Polyoma virus	Serum	ELISA	-	0/15	-	-	0/14
Reovirus 3 (REO 3)	Serum	MFI	0/16	0/15	0/16	0/14	0/112
Rotavirus (EDIM)	Serum	MFI	0/16	0/15	0/16	0/14	0/111
Sendai virus	Serum	MFI	0/16	0/15	0/16	0/14	0/112
BACTERIA & MYCOPLASMA							
Bordetella bronchiseptica	Oropharynx	Culture	0/22	0/20	0/20	0/19	0/156
CAR bacillus	Serum	ELISA	0/16	-	-	0/14	0/56
Citrobacter rodentium	Intestine or feces	Culture	0/109	0/92	0/81	0/80	0/808
Clostridium piliforme	Serum	ELISA	-	0/15	0/16	-	0/56
Corynebacterium bovis	Oropharynx/skin	Culture	0/23	0/20	0/20	0/24	0/156
Corynebacterium kutscheri	Oropharynx	Culture	0/22	0/20	0/20	0/19	0/156
Helicobacter spp.	Intestine or feces	PCR	0/06	0/06	0/06	0/06	0/48
Mycoplasma pulmonis	Serum	MFI	0/16	0/15	0/16	0/14	0/112
Pasteurella pneumotropica	Oropharynx	Culture	0/22	0/20	0/20	0/19	0/156
Salmonella spp.	Intestine or feces	Culture	0/109	0/92	0/81	0/80	0/808
Streptobacillus moniliformis	Oropharynx	Culture	0/22	0/20	0/20	0/19	0/156

The Jackson Laboratory
600 Main Street
Bar Harbor, ME 04609
1-800-422-6423

For additional details regarding our health monitoring program and shipping policy see www.jax.org/jaxmice/health

Organism	Sample Tested	Test Method	Oct 17 '11	Sep 5 '11	Jul 25 '11	Jun 13 '11	Previous 12 months
PARASITES & PROTOZOA							
Encephalitozoon cuniculi	Serum	MFI	0/16	0/15	0/16	0/14	0/84
Fleas	Fur	Visual	0/06	0/06	0/06	0/06	0/48
Fur mites, lice	Fur	Stereoscope	0/06	0/06	0/06	0/06	0/48
Follicle mites	Subcutis	Visual	0/22	0/20	0/20	0/19	0/156
Pinworms	Cecum	Visual	0/06	0/06	0/06	0/06	0/48
Opportunistic protozoa (e.g., Giardia, Spironucleus)	Intestine	Micro	0/06	0/06	0/06	0/06	0/48
Roundworms and other helminths	Intestine	Visual	0/06	0/06	0/06	0/06	0/48
Tapeworms	Intestine	Visual	0/06	0/06	0/06	0/06	0/48

OTHER ORGANISMS MONITORED (SHIPPING NOT STOPPED) -

Most of these organisms are excluded from most Repository barriers. When an excluded organism is found an investigation is undertaken to identify and eliminate all infected mice from the barrier. Positive results - including results from investigations - are noted in this report, but shipping from the area is not suspended.

Organism	Sample Tested	Test Method	Oct 17 '11	Sep 5 '11	Jul 25 '11	Jun 13 '11	Previous 12 months
Klebsiella pneumoniae	Oropharynx, intestine, or feces	Culture	0/109	0/92	0/81	0/80	0/808
Klebsiella spp. other than *K. pneumoniae*	Oropharynx, intestine, or feces	Culture	0/109	0/92	0/81	0/80	0/808
Nonpathogenic protoza (e.g., Trichomonads)	Intestine	Micro	0/06	0/06	0/06	0/06	0/48
Pneumocystis murina	Lung	PCR	0/06	0/05	0/04	0/05	0/44
Pseudomonas spp.	Intestine or feces	Culture	0/109	0/92	0/81	0/80	0/808
Staphylococcus aureus	Oropharynx	Culture	0/22	0/20	0/20	0/19	0/156
Streptococcus spp.	Oropharynx	Culture	0/22	0/20	0/20	0/19	0/156

James R. Fahey, MS, PhD, DVM, DACVM
Chief of Diagnostic Services

The Jackson Laboratory
600 Main Street
Bar Harbor, ME 04609
1-800-422-6423

For additional details regarding our health monitoring program and shipping policy see www.jax.org/jaxmice/health

AX-12

Health status information for *Alox15*$^{-/-}$ at Berlin.

CHARITÉ CAMPUS BENJAMIN FRANKLIN

Charité | Campus Benjamin Franklin | Forschungseinrichtung für Experimentelle Medizin (FEM) | Krahmerstr. 6 | 12207 Berlin

Health Monitoring in Accordance with FELASA Recommendations
BfR-FEM Berlin, Unit 8 R E04.05, E04.06, E04.07, E04.08,E04.09

Species Mouse

	Test frequency	Latest test results Juni -11	Previous results März -11	Testing laboratory	Test Method	Historicl results (<18 months)
Viruses:		n=10	N=10			
Mouse hepatitis virus (MHV)	3 months	NEG	NEG	GIM	IFA	NEG
Ectromelia virus	annually	NT	NEG	GIM	IFA	NEG
Mouse rota virus (EDIM)	3 months	NEG	NEG	GIM	IFA	NEG
Lymphocytic choriomeningitis virus	annually	NT	NEG	GIM	IFA	NEG
Mouse adenovirus type 1 (K87)	annually	NT	NEG	GIM	IFA	NEG
Mouse cytomegalo virus (MCMV)	annually	NT	NEG	GIM	IFA	NEG
Mouse Parvoviruses (MPV)	3 months	NEG	NEG	GIM	IFA	NEG
Minute virus of mice (MVM)	3 months	NEG	NEG	GIM	IFA	NEG
Pneumonia virus of mice (PVM)	3 months	NEG	NEG	GIM	IFA	NEG
Sendai virus	3 months	NEG	NEG	GIM	IFA	NEG
Theiler´s murine encephalomyelitis virus (TMEV)	3 months	NEG	NEG	GIM	IFA	NEG
Reovirus type 3	annually	NT	NEG	GIM	IFA	NEG
Bacteria, mycoplasma and fungi				GIM		
Citrobacter rodentium	3 months	NEG	NEG	GIM	CULT	NEG
Clostridium piliforme (Tyzzers´s disease)	3 months	NEG	NEG	GIM	IFA	NEG
Corynebacterium kutscheri	3 months	NEG	NEG	GIM	CULT	NEG
Mycoplasma spp.	3 months	NEG	NEG	GIM	IFA	NEG
Pasteurellaceae **	3 months	NEG	NEG	GIM	CULT/IFA	POS
Pasteurella pneumotropica	3 months	NEG	NEG	GIM	IFA	NEG
Salmonella spp.	3 months	NEG	NEG	GIM	CULT	NEG
Streptococci β-haemolytic (not group D)	3 months	NEG	NEG	GIM	CULT	NEG
Streptococcus pneumoniae	3 months	NEG	NEG	GIM	CULT	NEG
Helicobacter spp.*	annually	NT	POS (6/10)	GIM	PCR	POS
H.bilis	annually	NT	NEG	GIM		NEG
H.hepaticus	annually	NT	NEG	GIM		NEG
H. typhlonius	annually	NT	NEG	GIM		NEG
Streptobacillus moniliformis	annually	NT	NEG	GIM	CULT	NEG
Additional organisms tested						
Pneumocystis carinii	annually	NT	NEG	GIM	IFA	
Parasites						
Endoparasites:	3 months	NEG	NEG	GIM	MICR	NEG
Aspiculuris spp.	3 months	NEG	NEG	GIM	MICR	NEG
Syphacia spp.	3 months	NEG	NEG	GIM	MICR	NEG
Coccidien	3 months	NEG	NEG	GIM	MICR	NEG
Giardia spp.	3 months	NEG	NEG	GIM	MICR	NEG
Spironucleus muris	3 months	NEG	NEG	GIM	MICR	NEG
Flagella	3 months	NEG	NEG	GIM	MICR	NEG
Arthropoda	3 months	NEG	NEG	GIM	MICR	NEG
Pathological lesions observed	3 months	NEG	NEG	GIM	PATH	NEG

* Helicobacter rappini (formerly known as Flexispira r.)
** actinobacillus muris

Abbreviations used in this report: CULT = Culture; ELISA = Enzyme Linked ImmunoSorbent Assay; IFA = Indirect Fluorescence Assay; MICR = Microscopy; PATH = Gross Pathology; PCR = Polymerase Chain Reaction; NT = Not tested
Abbreviations used for Laboratories: GIM = GIM Gesellschaft für innovative Mikroökologie mbH, Waldheimstrasse 47, 14552 Berlin.
Data are expressed positive (POS) or negative (NEG)

Health status information for *Alox15*$^{-/-}$ at Cardiff, Nov 2010.

Surrey Diagnostics

Surrey Diagnostics Ltd..
PO Box 156, Cranleigh, GU6 8ZU T +44 (0)1483 26830
M 07778 010184 F +44 (0)1483 266537 E info@sdl.uk.n

www.sdl.uk.net

Client : Deborah Adams, University of Wales, Heath Park, Cardiff
Lab No. : 10/9192-9193
Sample Details : 2 Adult Female CD1/GA Mice from Room 213

Mouse Report	Test Frequency	Current Test Date	Current Test Results	Testing Laboratory	Test Method
Serology					
Minute Virus of Mice	3 months	2 Nov 2010	0/2	SDL	MFIA
Mouse Hepatitis Virus	3 months	2 Nov 2010	0/2	SDL	MFIA
Pneumonia Virus of Mice	3 months	2 Nov 2010	0/2	SDL	MFIA
Reovirus Type III	3 months	2 Nov 2010	0/2	SDL	MFIA
Mouse Encephalomyelitis Virus	3 months	2 Nov 2010	0/2	SDL	MFIA
Sendai Virus	3 months	2 Nov 2010	0/2	SDL	MFIA
Epizootic Diarrhoea of Infant Mice	3 months	2 Nov 2010	0/2	SDL	MFIA
Mouse Parvovirus	3 months	2 Nov 2010	0/2	SDL	MFIA
Mouse Norovirus	3 months	2 Nov 2010	**2/2**	SDL	MFIA
Clostridium piliforme	3 months	2 Nov 2010	0/2	SDL	MFIA
Mycoplasma pulmonis	3 months	2 Nov 2010	0/2	SDL	MFIA
Lymphocytic Choriomeningitis Virus	12 months	2 Nov 2010	0/2	SDL	MFIA
Hantaan Virus	12 months	2 Nov 2010	0/2	SDL	MFIA
Ectromelia Virus	12 months	2 Nov 2010	0/2	SDL	MFIA
Mouse Cytomegalovirus	12 months	2 Nov 2010	0/2	SDL	MFIA
Mouse Adenovirus 1	12 months	2 Nov 2010	0/2	SDL	MFIA
Mouse Adenovirus 2	12 months	2 Nov 2010	0/2	SDL	MFIA
Polyoma Virus	12 months	2 Nov 2010	0/2	SDL	MFIA
K Virus	12 months	2 Nov 2010	0/2	SDL	MFIA
Mouse Hepatitis Virus PCR	12 months	2 Nov 2010	0/2	SDL	RT-PCR
Parasitology					
INTESTINAL PROTOZOA	3 months	2 Nov 2010	0/2	SDL	MIC
FAECAL OVA	3 months	2 Nov 2010	0/2	AX/SDL	FLOT/MIC
HELMINTHS	3 months	2 Nov 2010	0/2	SDL	MIC
ATHROPODS	3 months	2 Nov 2010	0/2	SDL	MIC
Bacteriology					
Bordetella bronchiseptica	3 months	2 Nov 2010	0/2	SDL	CULT
Citrobacter rodentium	3 months	2 Nov 2010	0/2	SDL	CULT
Corynebacterium kutscheri	3 months	2 Nov 2010	0/2	SDL	CULT
Helicobacter species	12 months	2 Nov 2010	**2/2**	SDL	PCR
Helicobacter hepaticus	On Request				
Helicobacter bilis	On Request				
Klebsiella species	3 months	2 Nov 2010	0/2	SDL	CULT
Pasteurellaceae	3 months	2 Nov 2010	0/2	SDL	CULT
Pneumocystis carinii	On Request				
Pseudomonas aeruginosa	3 months	2 Nov 2010	0/2	SDL	CULT
Salmonella species	3 months	2 Nov 2010	0/2	SDL	CULT
Staphylococcus aureus	3 months	2 Nov 2010	0/2	SDL	CULT
Streptobacillus moniliformis	3 months	2 Nov 2010	0/2	SDL	CULT
Streptococcus pneumoniae	3 months	2 Nov 2010	0/2	SDL	CULT
B-haemolytic Streptococci	3 months	2 Nov 2010	0/2	SDL	CULT
Yersinia species	3 months	2 Nov 2010	0/2	SDL	CULT
Necropsy					
External Lesions/Abnormalities	3 months	2 Nov 2010	0/2	SDL	MIC
Internal Lesions/Abnormalities	3 months	2 Nov 2010	0/2	SDL	MIC

Parasitology Notes :

MFIA = Multiplexed Immuno-Fluorescent Assay, PCR= Polymerase Chain Reaction, CULT = Culture, MIC = Microscopy, FLOT = Faecal Flotation
SDL = Surrey Diagnostics Ltd, AX = Axiom Veterinary Services, BD = Bio-Doc

Health status information for *Alox15*$^{-/-}$ at Cardiff, May 2011

Surrey Diagnostics

Surrey Diagnostics Ltd..
PO Box 156, Cranleigh, GU6 8ZU T +44 (0)1483
M 07778 010184 F +44 (0)1483 266537 E info@

www.sdl.uk.net

Client : Deborah Adams, University of Wales, Cardiff
Lab No. : 11/4038-4039
Sample Details : 2 Adult Female Mice from Heath Park (CD1 + GA), Room 213 Conventional

Mouse Report	Test Frequency	Current Test Date	Current Test Results	Testing Laboratory	Test Method
Serology					
Minute Virus of Mice	3 months	4 May 2011	0/2	SDL	MFIA
Mouse Hepatitis Virus	3 months	4 May 2011	0/2	SDL	MFIA
Pneumonia Virus of Mice	3 months	4 May 2011	0/2	SDL	MFIA
Reovirus Type III	3 months	4 May 2011	0/2	SDL	MFIA
Mouse Encephalomyelitis Virus	3 months	4 May 2011	0/2	SDL	MFIA
Sendai Virus	3 months	4 May 2011	0/2	SDL	MFIA
Epizootic Diarrhoea of Infant Mice	3 months	4 May 2011	0/2	SDL	MFIA
Mouse Parvovirus	3 months	4 May 2011	0/2	SDL	MFIA
Mouse Norovirus	3 months	4 May 2011	**2/2**	SDL	MFIA
Clostridium piliforme	3 months	4 May 2011	0/2	SDL	MFIA
Mycoplasma pulmonis	3 months	4 May 2011	0/2	SDL	MFIA
Lymphocytic Choriomeningitis Virus	12 months	4 May 2011	0/2	SDL	MFIA
Hantaan Virus	12 months	4 May 2011	0/2	SDL	MFIA
Ectromelia Virus	12 months	4 May 2011	0/2	SDL	MFIA
Mouse Cytomegalovirus	12 months	4 May 2011	0/2	SDL	MFIA
Mouse Adenovirus 1	12 months	4 May 2011	0/2	SDL	MFIA
Mouse Adenovirus 2	12 months	4 May 2011	0/2	SDL	MFIA
Polyoma Virus	12 months	4 May 2011	0/2	SDL	MFIA
K Virus	12 months	4 May 2011	0/2	SDL	MFIA
Mouse Hepatitis Virus PCR	12 months	4 May 2011	0/2	SDL	RT-PCR
Parasitology					
INTESTINAL PROTOZOA	3 months	4 May 2011	**2/2**	SDL	MIC
FAECAL OVA	3 months	4 May 2011	0/2	AB/SDL	FLOT/MIC
HELMINTHS	3 months	4 May 2011	0/2	SDL	MIC
ATHROPODS	3 months	4 May 2011	0/2	SDL	MIC
Bacteriology					
Bordetella bronchiseptica	3 months	4 May 2011	0/2	SDL	CULT
Citrobacter rodentium	3 months	4 May 2011	0/2	SDL	CULT
Corynebacterium kutscheri	3 months	4 May 2011	0/2	SDL	CULT
Helicobacter species	12 months	4 May 2011	**2/2**	SDL	PCR
Helicobacter hepaticus	On Request				
Helicobacter bilis	On Request				
Klebsiella species	3 months	4 May 2011	0/2	SDL	CULT
Pasteurellaceae	3 months	4 May 2011	0/2	SDL	CULT
Pneumocystis carinii	On Request				
Pseudomonas aeruginosa	3 months	4 May 2011	0/2	SDL	CULT
Salmonella species	3 months	4 May 2011	0/2	SDL	CULT
Staphylococcus aureus	3 months	4 May 2011	0/2	SDL	CULT
Streptobacillus moniliformis	3 months	4 May 2011	0/2	SDL	CULT
Streptococcus pneumoniae	3 months	4 May 2011	0/2	SDL	CULT
B-haemolytic Streptococci	3 months	4 May 2011	0/2	SDL	CULT
Yersinia species	3 months	4 May 2011	0/2	SDL	CULT
Necropsy					
External Lesions/Abnormalities	3 months	4 May 2011	0/2	SDL	MIC
Internal Lesions/Abnormalities	3 months	4 May 2011	0/2	SDL	MIC

Parasitology Notes : Chilomastix sp found.

MFIA = Multiplexed Immuno-Fluorescent Assay, PCR= Polymerase Chain Reaction, CULT = Culture, MIC = Microscopy, FLOT = Faecal Flotation
SDL = Surrey Diagnostics Ltd, AB = Abbey Veterinary Services, BD = Bio-Doc

Friedrich-Alexander-Universität Erlangen-Nürnberg

FRANZ-PENZOLDT-ZENTRUM
Tierhaltung

Dr. med. vet. Roland Jurgons

Tel.: +49 9131 85-23506, -23500
Fax: +49 9131 85-23502
E-mail: Roland.Jurgons@ze.uni-erlangen.de

Health monitoring (according to FELASA recommendations)

Facility: Franz-Penzoldt-Zentrum Universität Erlangen-Nürnberg Palmsanlage 5 91054 Erlangen **IVC-Unit Sentinels** **Species:** Mice **Strain:** Balb/c	**Frequency** **quarterly**	**Latest results** **04/11**	**History** (kumulativ) **>18 month**	**Laboratory**
Viruses				
Ectromelia virus		0/48 ELISA	0/ 285	Mfd Diagnostics
Lymphocytic choriomeningitis virus		0/48 ELISA	0/ 285	Mfd Diagnostics
Minute virus of mice		0/48 ELISA	0/ 295	Mfd Diagnostics
Mouse adenovirus type 1 (MAd FL)		0/48 ELISA	0/ 285	Mfd Diagnostics
Mouse adenovirus type 2 (MAd K87)		0/48 ELISA	0/ 285	Mfd Diagnostics
Mouse cytomegalovirus		0/48 ELISA	0/ 285	Mfd Diagnostics
Mouse hepatitis virus		0/48 ELISA	0/ 295	Mfd Diagnostics
Mouse parvovirus		0/48 ELISA	0/ 295	Mfd Diagnostics
Mouse rotavirus (EDIM)		0/48 ELISA	0/ 295	Mfd Diagnostics
Pneumonia virus of mice		0/48 ELISA	0/ 295	Mfd Diagnostics
Reovirus type 3		0/48 ELISA	0/ 295	Mfd Diagnostics
Sendai virus		0/48 ELISA	0/ 295	Mfd Diagnostics
Theiler's murine encephalomyelitis virus		0/48 ELISA	0/ 295	Mfd Diagnostics
Mouse Norovirus (MNV)		***35/48 ELISA***	**151/ 259**	Mfd Diagnostics
Bacteria, Mycoplasma and Fungi				
Citrobacter rodentium		0/48 Culture	0/ 295	Mfd Diagnostics
Clostridium piliforme		0/48 ELISA	0/ 285	Mfd Diagnostics
Corynebacterium kutscheri		0/48 Culture	0/ 295	Mfd Diagnostics
Helicobacter spp		***10/48* PCR**	***31/ 285***	Mfd Diagnostics
Helicobacter hepaticus		0/48 PCR	***4/ 285***	Mfd Diagnostics
Mycoplasma spp		0/48 ELISA	0/ 285	Mfd Diagnostics
Pasteurella spp		0/48 Culture	***3*/ 295***	Mfd Diagnostics
Pneumocystis carinii		nt	0/ 7	Mfd Diagnostics
Pseudomonas aeruginosa		0/48 Culture	***20/ 285***	Mfd Diagnostics
Salmonella spp		0/48 Culture	0/ 295	Mfd Diagnostics
Staphylococcus aureus		***2/48 Culture***	***26/ 285***	Mfd Diagnostics
Streptobacillus moniliformis		0/48 Culture	0/ 285	Mfd Diagnostics
Streptococci β-haemolytic (not group D)		0/48 Culture	0/ 295	Mfd Diagnostics
Streptococcus pneumoniae		0/48 Culture	0/ 295	Mfd Diagnostics
Parasites				
Ectoparasites		0/48 Microscopy	0/ 295	Mfd Diagnostics / GIM
Endoparasites				
Entamoeba spp		0/48 Microscopy	***8/ 295***	Mfd Diagnostics / GIM
Tritrichomonas spp		***13/48 Microscopy***	***88/ 295***	Mfd Diagnostics / GIM
Chilomastix spp		***8/48 Microscopy***	***55/ 295***	Mfd Diagnostics / GIM
Pathological Lesions				
External		0/48 Path./ Hist.	0/ 295	Mfd Diagnostics
Necropsy		0/48 Path./ Hist.	0/ 295	Mfd Diagnostics

* Pasteurella pneumotropica, Room 1.291

Erlangen, 23.05.2011

Health status information for *Alox15*$^{-/-}$ at Philadelphia

Tests conducted by: Charles River Diagnostics,
251 Ballardvale Street, Wilmington, MA 01887, USA.
Phone: 800-338-9680.

Accession #s: 2005-032298, 2007-045849
Hard copy originals are on file in Philadelphia and Cardiff.

Pathogen	28th Sept 2005	18th Dec 2007
ELISA SEND	-	-
ELISA PVM	-	-
ELISA MHV	-	-
ELISA GDVII	-	-
ELISA REO	-	-
ELISA MPUL	-	-
ELISA LCMV	-	-
ELISA ECTRO	-	-
ELISA K	-	-
ELISA POLY	-	-
ELISA MAV 1& 2	-	-
ELISA EDIM	-	-
ELISA MPV	-	-
ELISA PARV NS1	-	-

Approved by Keith Provencher (2005), Rosanilis Tejada (2007)